

Genes, Development and Disease *Group*

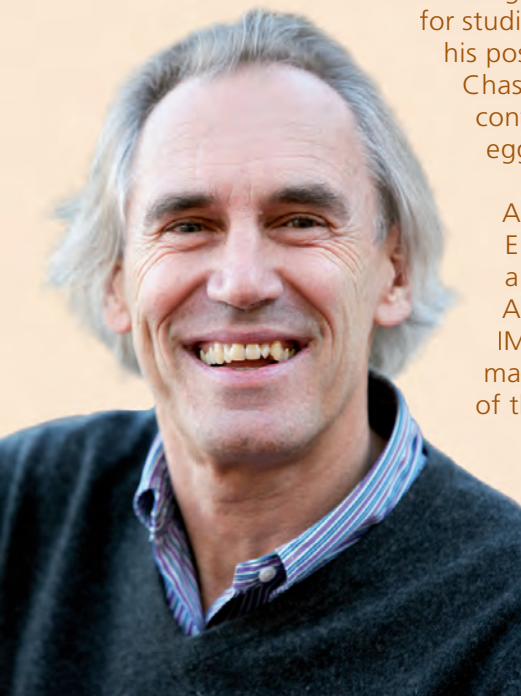
Summary

The major focus of our studies is the analysis of gene function in normal and pathological conditions, e.g. in tumour development, using the mouse as a model organism, but also human samples. Specifically, the functions of the AP-1 (Fos/Jun) transcription factor complex in regulating cell proliferation, differentiation and cell death are being investigated in transgenic mouse models for inflammatory processes and common human diseases such as psoriasis, fibrosis, bone loss and cancer. The major goal is to analyse the molecular pathways leading to these diseases and to identify novel therapeutic approaches.

Strategic Goals

- Elucidate a causal link between AP-1 (Fos/Jun) expression and inflammation as well as cancer using inducible mutant mouse models
- Develop and characterise mouse models for human diseases such as osteoporosis, fibrosis and psoriasis and their use for preclinical studies
- Establish human ES and iPS cells for gene expression studies and efficient differentiation e.g. into cartilage for tissue regeneration

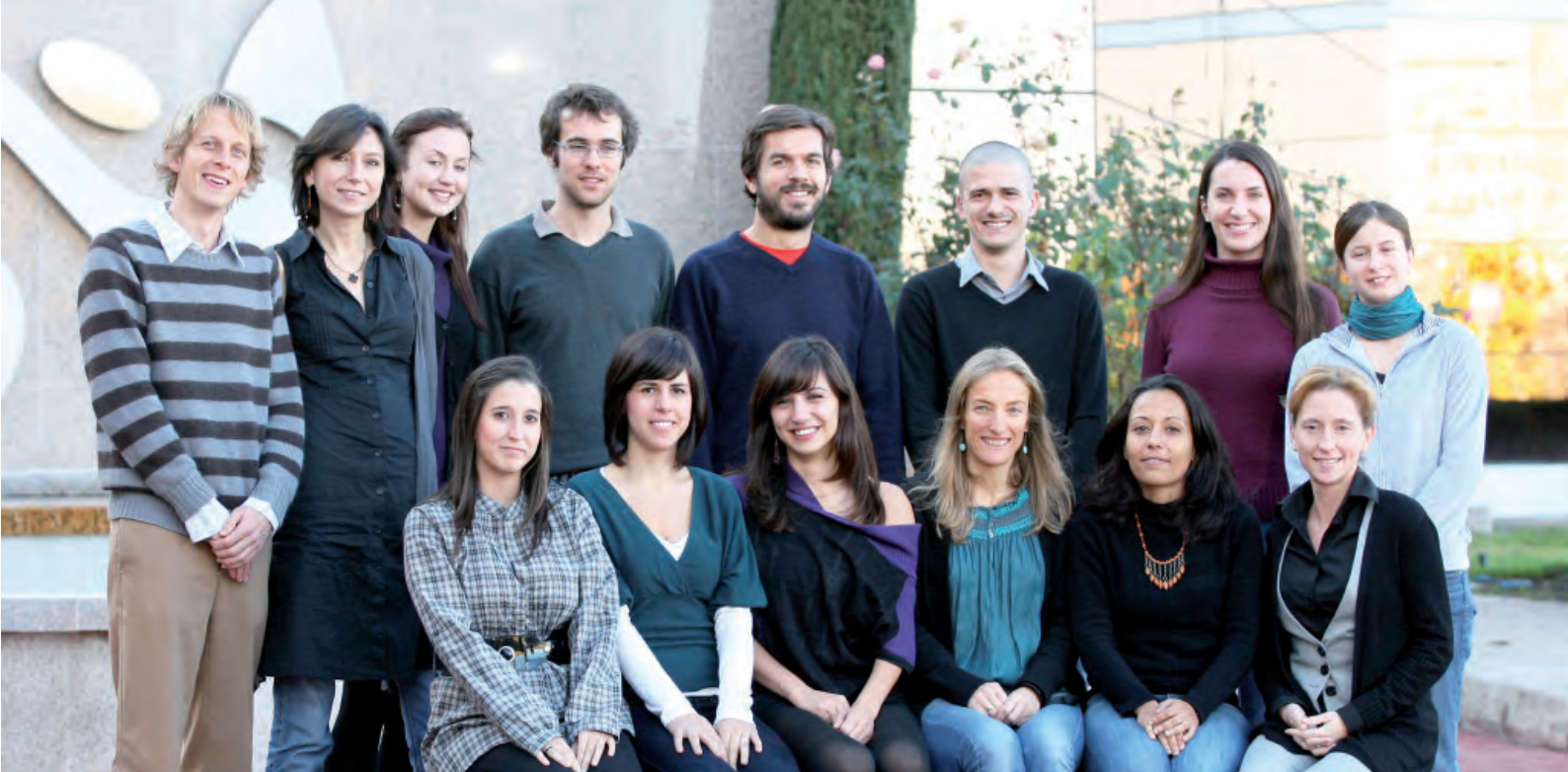
Erwin F. Wagner *Group Leader*



Erwin Wagner received his university education in Austria and obtained his PhD in 1978 for studies on the regulation of gene expression during bacterial T1 infection. Further to his postdoctoral training in Innsbruck, he joined the laboratory of B. Mintz at the Fox Chase Cancer Centre in Philadelphia, USA, in 1979 where he worked on the genetic control of mouse development by developing micro-injection of DNA into fertilised eggs and gene transfer technologies into stem cells and mice.

After spending four years in Philadelphia, he became a Group Leader at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, in 1983 and then joined the Research Institute of Molecular Pathology (IMP) in Vienna, Austria, in 1988 as a Senior Scientist and Founding Member. He worked at the IMP for 20 years heading a Senior Research Group working on gene functions in mammalian development and oncogenesis. He was also appointed Deputy Director of the IMP from 1997 to 2008.

He and his lab moved to the CNIO in 2008 where he is currently Vice Director and also Director of the newly founded BBVA Foundation - CNIO Cancer Cell Biology Programme as well as Head of the Genes, Development and Disease Group.



Staff scientists: Latifa Bakiri, Aline Bozec, Juan Guinea-Viniegra, María Jiménez, Helia B. Schönthaler. **Post-doctoral fellows:** Guillaume Beranger, Martin Kristian Thomsen (since July), Özge Uluçkan (since September). **Graduate students:** Eva Briso de Montiano, Sebastian Hasenfuss. **Technicians:** Holly A. Gefroh, Jessica Rubio, Stefanie Wculek (since May), Stefanie Wurm (since July).

Highlights

Fos/AP-1 – functions in bone development

Fos proteins are key regulators of bone development. Transgenic mice over-expressing c-Fos develop osteoblastic bone tumours, whereas mice lacking c-Fos are osteopetrotic (op) and lack bone-resorbing osteoclasts (Figure 1).

We have addressed the relevance of post-translational modifications of Fos in bone development and pathogenesis. ERK and the ERK-dependent kinase RSK2 can phosphorylate Fos on serines 362 and 374. RSK2 is essential for the development of Fos-dependent osteosarcomas *in vivo*. We generated Fos-mutant knock-in mice in which the

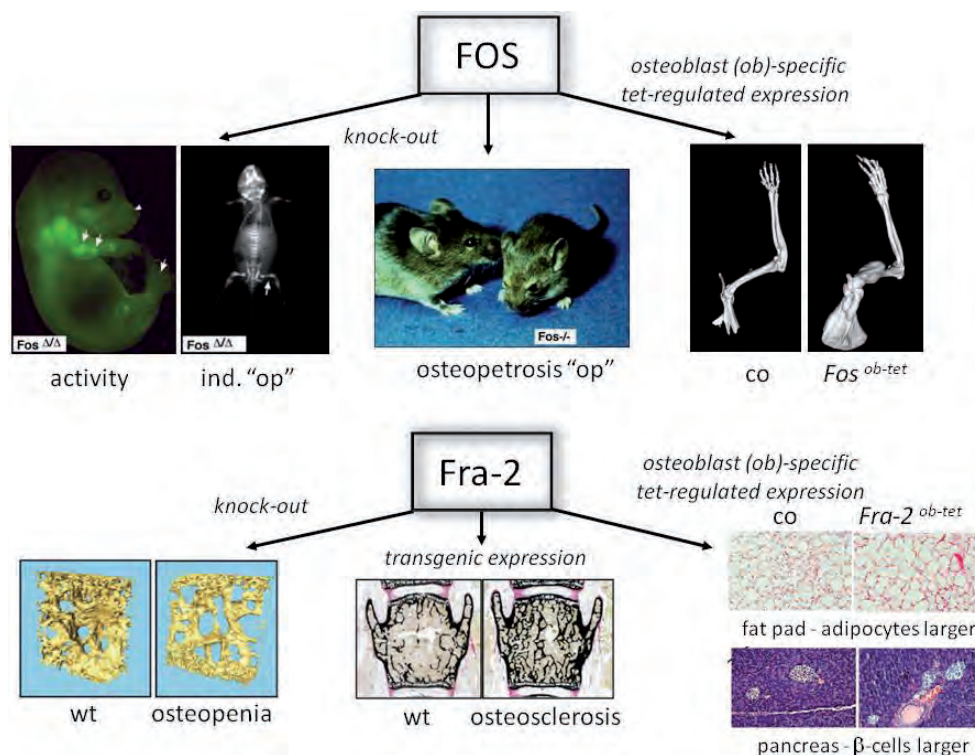


Figure 1: Functional analysis of Fos and Fra-2 proteins in development and disease.

endogenous wild-type Fos allele was replaced by an allele where serines 362 and 374 are mutated to alanines (*Fos^{AA}*). Recent results show that Fos C-terminal phosphorylation is dispensable for skeletogenesis, but required for both osteoclastogenesis *in vitro* and normal bone homeostasis in adult mice.

Recently we identified TGFBI (TGFβ-induced gene) as a potential c-Fos target gene implicated in tumour development. We are currently investigating the function of TGFBI in bone physiology and osteosarcoma/rhabdomyosarcoma formation using *in vitro* approaches in mouse and human cells as well as genetically modified mice.

Using loss and gain of function approaches in mice, we analysed the functions of the Fos-related protein Fra-1 and Fra-2. Fra-2 is essential for postnatal mouse development and maintenance of proper bone mass. Mutant newborns die with severe osteopenia and giant osteoclasts (Figure 1). Moreover, transgenic mice over-expressing Fra-2 have increased bone mass and develop pulmonary

fibrosis. Interestingly, expression of Fra-2 in osteoblasts leads to increased osteoblast differentiation at the expense of adipocytes. This correlates with increased osteocalcin and decreased adiponectin secretion by osteoblasts. Moreover, mice lacking Fra-2 expression in osteoblasts also display a metabolic phenotype (Figure 1). These novel results show that osteoblast-specific expression of Fra-2/AP-1 is implicated in the endocrine regulation of energy metabolism by bone.

Switchable AP-1 transgenes

To better define the role of AP-1 proteins in general physiology we have recently established efficient, switchable AP-1 transgenes (see also report by Sagrario Ortega). We have employed a highly efficient Flp-mediated transgene integration strategy using Embryonic Stem (ES) cells to generate a collection of doxycycline-switchable AP-1 alleles. These alleles allow temporal, dose-dependent, reversible, as well as tissue-specific expression of AP-1 monomers or forced dimers *in vitro* and *in vivo*. For example, X-ray analyses of switchable c-Fos mice revealed a very

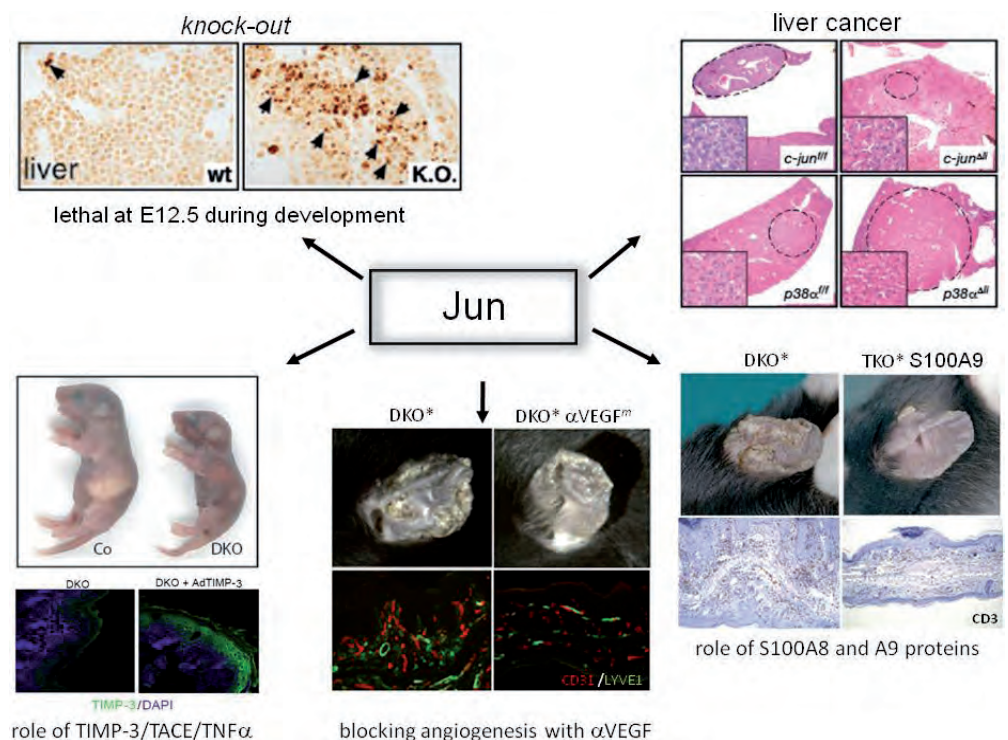


Figure 2: Functional analysis of Jun proteins in liver and inflammatory skin disease.

fast increase in bone mineral density and the appearance of tumours within or in contact with the bone matrix upon ectopic c-Fos expression (Figure 1). On the other hand, inducible c-Fos expression in the epidermis of the skin leads to a hyperproliferation and Fos appears to act as a co-factor in skin cancer development.

Jun/Fos – role in proliferation, differentiation and apoptosis

Genetic strategies are employed to investigate the functions of Jun/AP-1 and MAPK proteins in liver cancer and inflammatory disease. The upstream activator of Jun, JNK1, plays an oncogenic role in liver carcinogenesis, while p38 α suppresses liver cancer development (Figure 2). During acute hepatitis, c-Jun mediates hepatocyte survival by regulating iNOS expression, thus protecting the liver from hypoxia and oxidative stress. We are currently analysing Jun's functions in hepatitis-associated carcinogenesis.

When JunB was inactivated in the epidermis mice were born healthy but developed a multiorgan disease likely caused by deregulated keratinocyte-derived G-CSF and IL-6 cytokines. In addition, employing an inducible mouse model, we have demonstrated in human patient samples that downregulation of

Jun proteins in keratinocytes can cause a psoriasis-like disease. Interestingly, when Jun and JunB were inactivated in the epidermis in a constitutive manner, mutant pups died due to a cytokine storm involving deregulation of TNF-TIMP3-TACE expression (Figure 2).

Mice developing this psoriasis-like disease are also being studied regarding the involvement of angiogenesis, e.g. VEGF expression and the role of the Jun target genes S100A8 and S100A9 in disease development (Figure 2). Finally, deletion of Fos in keratinocytes did not induce any obvious skin phenotype under normal conditions, while keratinocytes subjected to stress differentiated prematurely due to activation of the p53/Notch1 pathway.

AP-1 function in mesodermal differentiation and tissue regeneration

We have started to establish efficient protocols to induce differentiation of murine and human ES cells into different mesodermal lineages, including chondro-, osteo- and vascular progenitor cells. We will perform functional studies of the AP-1 (Fos/Jun) complex during murine and human ES cell differentiation and also use *in vitro* differentiated, genetically altered progenitor cells for tissue engineering.

Publications

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Julien M, Khoshniat S, Lacreusette A, Gatius M, Bozec A, Wagner EF, Wittrant Y, Masson M, Weiss P, Beck L, Magne D, Guicheux J (2009). Phosphate-dependent regulation of MGP in osteoblasts: role of ERK1/2 and Fra-1. *J Bone Miner Res* 24, 1856-1868.

Awards and Recognition

International Research Prize from the Austrian Society of Bone and Mineral Research